

# **ROLE OF CD10 EXPRESSION IN COLORECTAL CARCINOMA AND PREMALIGNANT LESIONS**

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## **OBJECTIVE:**

1. To study the immunohistochemical expression of CD10 in colorectal carcinoma and in premalignant lesion.
2. To correlate the expression of CD10 in tumour cells and stromal cells with various clinico pathologic variables.

## **INTRODUCTION:**

Continuous and bilateral molecular interaction between normal epithelial cells and stromal cells is disrupted by several factors secreted by tumour cells or by stromal cells under the influence of tumour cells.

CD 10 is a matrix metallopeptidases involved in carcinogenesis through the release of bioactive molecules that stimulate invasion, extracellular matrix degradation, inhibition of apoptosis and promotion of angiogenesis and immune response modulation.

## **MATERIAL AND METHODS:**

The study was conducted during the period of August 2016 to August 2017. It was carried out in specimens obtained from patients with confirmed histopathological diagnosis of colorectal adenomas and adenocarcinomas. The study was approved by the Ethical committee of Government Stanley Medical College and hospital.

**STUDY DESIGN:** Retrospective and comparative study.

## **STUDY POPULATION:**

**CASES:** The study sample comprised of 60 colorectal adenoma and adenocarcinoma patients. Cases were chosen from the Department of Surgical Gastroenterology, Department of Surgery, Government Stanley Medical College and hospital. Age, sex, tumor site, histological grade and tumor stage were obtained for all cases. All the 60 cases were screened for CD 10 expression through immunohistochemical assay.

## **INCLUSION CRITERIA:**

Patients with colorectal adenomas and adenocarcinomas.

## **EXCLUSION CRITERIA:**

Chemotherapy and/or radiotherapy prior to sampling.

Recurrent and metastatic adenocarcinomas.

Cancer types other than adenocarcinomas.

#### **METHOD OF DATA COLLECTION:**

A total of 60 cases, 30 each of colorectal adenoma and colorectal adenocarcinoma were studied at random and selected applying the inclusion and exclusion criteria. The tissues so obtained were processed and sections were cut at 5 microns. Hematoxylin and eosin stained sections were stained and analysed. The clinico-pathological characteristics including age, sex, tumor site, histological diagnosis (Adenoma/adenocarcinoma), histological grade (adenomas- low grade/high grade dysplasia ; adenocarcinoma – low/high) and adenocarcinoma stage were obtained for all the cases.

#### **METHOD OF TISSUE PREPARATION:**

10% neutral buffered formalin was used for fixing the specimens. The tissues were processed in various grades of alcohol and xylene. Paraffin blocks were prepared and sections of 5 micron thickness were cut in semiautomatic microtome. Staining was done by haematoxylin and eosin. Suitable blocks were chosen for immunohistochemistry.

#### **IMMUNOHISTOCHEMICAL STAINING FOR CD 10:**

IHC was performed on the selected blocks for CD10 immunohistochemistry. Slides coated with chrome alum were used. Sections were subjected to antigen retrieval using pressure cooker technique using TRIS EDTA (pH 9.2) buffer solution and then treated by HRP (horseradish peroxidase) polymer technique.

#### **HRP POLYMER TECHNIQUE**

The coated slides were taken through the following stages -

1. Overnight incubation (first at 70 degree Celsius for one hour, then at 40-45 degree Celsius for overnight).
2. Xylene-2 changes, 15 minutes each.
3. Graded alcohol-first with absolute alcohol - 2 changes, 5 minutes each. Then for 3 minutes with 90% alcohol and finally for 3 minutes with 70% alcohol.
4. Distilled water rinse for 2-5 minutes.
5. Antigen retrieval with pressure cooker.
6. Wash with tap water gradually.
7. TRIS buffer wash for 5 minutes, 2 changes.
8. Treatment with peroxidase block-for inhibiting endogenous peroxidases in the tissue for 10 minutes.
9. TRIS buffer wash for 5 minutes, 2 changes.
10. Application of primary antibody for 45 minutes.
11. TRIS buffer wash for 5 minutes, 2 changes.
12. Application of super enhancer for 15 minutes which enhances the final reaction product by increasing the sensitivity of antigen antibody reaction.
13. TRIS buffer wash for 5 minutes, 2 changes.

14. Application of secondary antibody from the goat with the tagged horseradish peroxidase enzyme for 15 minutes.
15. TRIS buffer wash for 5 minutes, 2 changes.
16. Application of DAB (Diaminobenzidine) chromogen for 5 minutes –this is cleaved by the enzyme to give the colored product at the antigen sites.
17. Wash in distilled water for 5 minutes.
18. The slides were counterstained with hematoxylin.
19. Air dried, dehydrated, cleared and mounted with DPX (Distyrenedibutyl phthalide in Xylol).

**SCORING CRITERIA FOR CD10:** Tumour CD10 was considered positive if more than 10% of tumour cells express fine to coarse cytoplasmic granules. Stromal CD10 was graded according to a 4 point scale based on percentage of positively stained area:

- 0- <10% positive tumour cells,
- +1 -10-25% positive tumour cells,
- +2 -25%-50% positive tumour cells,
- +3 ->50% positive tumour cells,

## **DISCUSSION:**

### **1. Age, gender and site distribution:**

The present study includes 60 cases which is comprised of 30 cases of colorectal adenomas and 30 cases of colorectal adenocarcinomas. The age group of patients included in the study varied from less than 20 years to more than 70 years with most of the patients being above 50 years of age. 40 % of the patients were males and 20 % were females. In most of the patients, the tumor was located in the rectum (31.67%), followed by descending colon (25%), Ascending colon (18.33%) and Rectosigmoid junction (6.67%).

### **2. Histological Type Distribution:**

Among the study participants, 30 (50.00%) were adenocarcinomas and 30 (50.00%) were adenomas.

### **3. Carcinoma grade and stage distribution :**

Out of the 30 adenocarcinomas that were studied, 10 cases were well differentiated, 10 cases were moderately differentiated and 10 cases were poorly differentiated. 2 (6.67%) were stage I, 12 (40.00%) were stage II, 12 (40.00%) belonged to stage III and 4 (13.33%) were stage IV.

### **4. Comparison of cd10 expression with age :**

In present study (N=60) mean tumour and stromal CD 10 in different age group of people <50 yrs and people >50 yrs were not statistically significant.

Wang-et al in 2005 observed in his study that comparison of CD 10 expression with age of the patient was statistically not significant. Jang –et al also showed no correlation between CD10 expression and age of the patient.

Iwase –et al also proved there was no statistical significance between CD 10 Expression and age of the patient.

### **5. Comparison of cd10 expression with gender:**

In the present study, 40 patients (66.67%) were male and 20 (33.33%) were female. Among the study participants, Mean Tumour CD10 in male was

48.78+37.23 and mean Tumour CD10 Median in female was 80.92+25.66. The difference in between mean Tumour cd10 expression and sex is statistically significant (P Value 0.021).

Wang et al and colleagues in their study conducted during 2005 found significant correlation between CD 10 expression by the tumor and gender .

Koga et al also showed that CD 10 expression by colorectal neoplasia did correlate with the gender.

Conversely Iwase et al. in their study concluded that no correlation existed between gender and CD 10 expression by colorectal adenomas and carcinomas .

#### **6. Tumour CD 10 Expression in adenomas vs adenocarcinomas (The adenoma-carcinoma sequence):**

In the present study, cd 10 expression gradually increased in adenomas from low grade dysplasia to high grade dysplasia and was maximally expressed in adenocarcinomas. The differences in mean CD 10 expression between adenomas and adenocarcinomas was statistically significant (P value less than 0.001).

This observation of gradually increased expression of CD10 in adenomas and significantly increased expression in adenocarcinomas makes CD10 a potentially exploitable target of anti-cancer therapy with maximal targeting of the tumor and minimal damage to the normal epithelium. Also the significant increase observed in CD10 expression from adenoma to adenocarcinoma suggests that CD 10 has an important role in colorectal tumorigenesis and malignant transformation of adenomas (the adenoma-carcinoma sequence).

The mean difference of Tumour CD10 and stromal CD10 expression in subjects with carcinoma and adenoma was 41.41. It is statistically significant (p value 0.001).

This result of the present study correlates with that of Jang et al<sup>5</sup> in 2013 stated that tumour CD 10 Expression significantly increased from 14% in low grade adenoma (3 cases out of 22 cases), to 22% in high grade adenomas (6 cases out of 27 cases) and 44% in invasive colorectal carcinoma (14 cases out of 32 cases) and this supports the involvement of CD10 in progression and carcinogenesis of colorectal carcinoma.

Wang et al<sup>63</sup> in 2013 reported there was progression in tumour CD10 Expression from 0.8% in low grade adenoma to 9.1% in high grade adenomas and 40% in invasive colorectal carcinoma.

Hirano et al<sup>7</sup> in 2012, Koga et al<sup>64</sup> in 2008, Iwase et al<sup>65</sup> in 2005 also supported that CD 10 Expression were reported more frequently in invasive phenotype rather than adenomas.

#### **7. Comparison between Tumour and Stromal CD10 expression and the depth of tumour invasion:**

The present study includes 30 cases of colorectal adenocarcinomas of which, 10 were seen in T2 depth of tumour (Tumour invades muscularis propria), 11 were seen in T3 depth of tumour (Tumour invades through the muscularis propria in to the pericolorectal tissue) and 9 were seen in T4 depth of tumour (Tumour penetrates the visceral peritoneum).

The difference in between Tumour CD 10 median and depth of invasion is statistically significant (P value less than 0.001). A significant correlation is observed between Tumour and Stromal CD10 expression and depth of invasion.

Hirano et al reached similar conclusions in their study on colorectal carcinomas which showed that CD 10 expression correlated significantly with depth of tumour invasion. The results of the present study are in agreement with, Koga et al who in their study conducted in 2008 comprising 48 cases of colorectal adenocarcinomas concluded that CD10 expression correlated with the depth of tumour invasion.

Likewise Ogawa et al<sup>66</sup> reported higher stromal CD10 expression in more invasive tumour. In contrast to the observations made in the present study, Jang et al in 2013 concluded that there was no correlation between CD10 expression and depth of tumour invasion.

#### **8. Comparison between Tumour and Stromal CD10 Expression with gross feature**

In our present study with study group of 30, mean difference of stromal CD 10 expression in ulceroproliferative growth across the group is statistically significant (p value-0.047). Ogawa et al<sup>66</sup> analyzed the expression of CD10 in the cell stroma and showed significantly more positivity in protruding lesion.

Waisberg et al also reported positivity for CD10 more in exophytic appearance of tumour.

Yao et al<sup>67</sup> in 2002 also supported the findings of significant CD 10 positivity high in proliferative lesion than in infiltrating lesion.

#### **9. Comparison between Tumour and Stromal CD10 expression and Nodal status of Patient.**

In our study group (N=30), the mean difference between the TUMOUR CD10 in subjects with Positive nodal status and in subjects with negative nodal status across the group is (26.25). It is statistically significant (P Value 0.049)

The mean difference between the STROMAL CD 10 in subjects with positive nodal status and in subjects with negative nodal status across the group is 30.38. It is statistically significant.

Bernescu<sup>68</sup> et al conducted a study in 2016 with 191 patients, cancer stages ranging from T1N0 to T3N2 and found CD 10 expression clinically significant in node positive cases.

**10. Comparison between Tumour and Stromal CD10 expression and stage of colorectal adenocarcinomas :** On comparing the CD10 expression between stage I and II adenocarcinomas and stage III adenocarcinomas it was found that the difference in mean CD10 expression between the two groups was not statistically significant showing that CD10 expression does not correlate with the stage of colorectal adenocarcinomas.

Fujita et al in 2007 also showed expression of CD 10 did not show any statistical significance with the clinical staging of colorectal adenocarcinoma

Waisberg et al in 2012 analysed the expression of CD 10 in various stage of colorectal carcinoma and found no positive correlation.

#### **CONCLUSION:**

The aim of the present study was to examine the expression of CD10, cell membrane metalloproteinase in colorectal neoplasia, its role in the transition sequence from adenoma to adenocarcinoma and its association with various clinicopathological characters of adenocarcinomas. The following are the conclusions of the present study

- CD 10 expression showed a significant increase from adenoma to adenocarcinoma. This signifies that CD10 plays an important role in all stages of the adenoma-carcinoma sequence, which includes the early event of adenoma formation from normal epithelium and its malignant transformation.
- The expression of CD10 showed significant correlation with Depth of tumour invasion and nodal status
- These results highlight the association of CD 10expression with malignant behaviour of colorectal adenocarcinomas and CD10 could prove to be a new biomarker for aggressiveness and prognostic information in these tumors.
- There was however no correlation between CD 10expression and the age and tumour stage
- The finding of gradually increasing CD 10 expression in adenoma to significantly higher expression in adenocarcinomas makes CD 10 an attractive and potential therapeutic target, which when implemented will result in maximal targeting of cancerous and also pre cancerous tissues with minimal damage to the surrounding normal mucosa.

#### REFERENCES:

1. Tissue expression of CD10 protein in colorectal carcinoma and its correlation with anatomorphological features of the tumour and with lymph node and liver metastasis.
2. CD 10 expression in colorectal carcinoma and premalignant lesions.